Effect of Dental Materials Calcium Hydroxide–containing Cement, Mineral Trioxide Aggregate, and Enamel Matrix Derivative on Proliferation and Differentiation of Human Tooth Germ Stem Cells

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Abstract

Introduction: Biocompatibility of pulp capping materials is important for successful use in dentistry. These materials should be nontoxic and permissive for proliferation and induction of odontogenic differentiation of pulp cells. The aim of our study was to evaluate the effects of enamel matrix derivative (EMD), mineral trioxide aggregate (MTA), and calcium hydroxide–containing cement (DYCAL) on proliferation and odontogenic differentiation of human tooth germ stem cells (hTGSCs) in which cells belonging to both pulp tissue and dental follicle exist. **Methods:** The 96-well plates, 24-well plates, and special chamber slides were coated with biomaterials for cell proliferation, differentiation, and scanning electron microscopy analysis. Odontogenic differentiation of hTGSCs was evaluated by analyzing mRNA expression of dentin sialophosphoprotein (DSPP) by real-time polymerase chain reaction expression analysis, measurement of alkaline phosphatase activity, and visualization of calcium depositions by von Kossa staining. **Results:** Our results demonstrate that EMD is the best material in terms of inducing differentiation and proliferation of hTGSCs. DYCAL was found to be toxic to hTGSCs; however, EMD-coated DYCAL showed less toxicity. EMD-coated MTA was not efficient at inducing proliferation and differentiation. **Conclusions:** Pulp capping materials come in direct contact with dental pulp cells; thus, they require comprehensive evaluation of interactions between cells and biomaterials. Therefore, we cultured hTGSCs, capable of odontogenic differentiation, on pulp capping materials directly. Our results suggest that combination of capping materials with EMD would increase the quality of capping by increasing biocompatibility of capping materials. *(J Endod 2011;37:650–656)*

Key Words

Calcium hydroxide-containing cement (DYCAL), enamel matrix derivative (EMD), human tooth germ stem cells, mineral trioxide aggregate (MTA), pulp capping

Recent advances in stem cell biology provide new strategies for regenerative endodontics. It has been shown that bone marrow stem cells, which are currently most widely used in clinical applications, are able to differentiate into odontoblasts and form hard tissue (1). On the other hand, dental stem cells including dental pulp cells (DPCs), dental follicle cells, and periodontal stem cells were isolated, characterized, and used in tooth tissue engineering (2). In the presence of signaling molecules transforming growth factor-beta (TGF-β), bone morphogenetic proteins BMP-2, BMP-4, BMP-7, and heme oxygenase-1 enzyme, DPCs differentiated into odontoblast cells (3–5). During treatment of exposed vital pulp, differentiation and proliferation of pulp cells are also affected dramatically by the interactions of DPCs and pulp capping materials. Calcium hydroxide–containing cement (DYCAL) is an antibacterial material that is routinely used as pulp capping agent. Induction of inflammation in clinical use is disadvantage of DYCAL (6). Mineral trioxide aggregate (MTA) has been shown to induce hard tissue formation within 2 weeks with limited inflammation (7). It was suggested that MTA increases dentin regeneration more effectively than calcium hydroxide (8) probably as a result of release of large numbers of Ca^{2+} ions (9) or inducing periodontal fibroblasts to secrete BMP-2 and TGF-β1 (10, 11). Enamel matrix derivative (EMD) has been reported to be very effective in regeneration of cementum, periodontal ligament, and bone (12). It was hypothesized that EMD exerts its therapeutic effect by providing an extracellular matrix that forms a more natural microenvironment for cells, stimulating cell attachment and differentiation (13). Biologically active molecules, present in EMD, do not cause severe allergic reactions except minimal inflammation (13, 14), but they increase proliferation of cells and increase hard tissue formation (15). In a recent study it was demonstrated that when MTA and EMD were applied to human DPCs together, the cells differentiated into odontoblast-like cells, suggesting a synergistic effect of 2 materials (16).

In clinical use, pulp capping materials are in direct contact with pulp tissue. Most of the studies investigating the effects of pulp capping materials on dental stem cells used plate inserts or conditioned medium obtained by incubation with materials. In our study, it was aimed to investigate the direct interaction between cells and the pulp capping materials by directly culturing the cells on the materials. We also tested the effects of combination of EMD with DYCAL and MTA on hTGSCs, which might be considered a new approach for pulp capping applications.

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